KTR

Final Report

TBR-0007-14

Silver Ion Sterilizer

Skin irritation test of silver ion sterilizer on rabbits

President of Korea Testing & Research Institute

Study Overview

Study Title: Skin irritation test of silver ion sterilizer on rabbits

Study Number: TBR-0007-14

Sponsor:

Name: CNL Co. Ltd.

Location: 1 Yeonseoidaegil, Heungeupmyeon, Wonju City, Gangwon-do (#304 Business Start-up and Childcare Center for the Disabled)

CEO: KIM, Kyeong Su

Person in charge: SONG, Cheoul Ki Position: Executive director

Contact: Tel. 033-764-2116 Fax. 033-764-2117

Test Facility:

Name: Health Care Research Laboratory of the Korea Testing & Research Institute (KTR)

Location: 12-63 Sandan-gil, Hwasun-eup, Hwasun-gun, Jeollanam-do

Operator in charge: PARK, Jong Il

Contact: Tel. 061-370-7810 Fax. 061-370-7777

Test Method:

-Ministry of Food & Drug Safety Announcement no. 2014-136 (July, 30, 2014), "Toxicity Test Standards for Drugs etc.", [Appendix 8] Topical toxicity test, ② Skin irritation test

LEE Jin Young, B. S. Date

Study Director

※ This is a report for the test substance provided by the sponsor.

Study Staffs

The following study staffs conducted the important testing steps of the study and reported the results thereof according to the standard operating guidelines of the Health Care Research Laboratory, KTR.

Staff in charge of the study:	CHEONG, Myeong Ho/B.S.
Staff in charge of preparing the test substance:	LEE, Jin Young/B.S.
Staff in charge of animal management:	KIM, Yong Wu/B.S.
Staff in charge of quarantine:	LEE, Jin Young/B.S.
Staff in charge of writing the report:	LEE, Jin Young/B.S.

Contents

1. Summary

In order to evaluate skin irritation on rabbits by silver ion water generated from a silver ion sterilizer, we applied the test substance on the rabbits' skin, and then at 24 hours and 72 hours after application of the test substance, we evaluated the mortality, general symptoms, weight changes, and skin irritation.

The result was as below.

-There were no animals that died due to the application of the test substance during the testing period.

-There were no special abnormal findings in all the animals.

-All the animals showed normal increase of weight during the testing period.

-No skin irritation was observed at 24 hours and 72 hours after application of the test substance.

-The evaluation result by Draize calculating method: Primary Irritation Index(P.I.I.) was '0.0'.

According to the above skin irritation test on rabbits, silver ion sterilizers are considered to be 'non-irritant' matter.

2. Introduction

This study was conducted to evaluate the skin irritation of silver ion sterilizers to rabbits.

This study was conducted with the approval from the Animal Ethics Committee of the Health Care Research Laboratory of the Korea Testing & Research Institute (KTR) based on the Animals Protection Act[enforcement Aug. 14 2014][Regulation no. 12051(partially amended on Aug. 13, 2013) and the Regulations on Animals for Testing[Enforcement Jul. 30, 2013][Regulation no. 11987(partially amended on Jul. 30, 2013)].

2.1 Test Schedule

Study initiation date	: September 30, 2014
Test initiation date	: October 6, 2014
Date of animal adoption	: October 6, 2014
Quarantine and Purification period	: October 6, 2014 ~ October 13, 2014
Date of waxing	: October 13, 2014
Date of application	: October 14, 2014
General symptom observation period	: October 14, 2-14 ~ October 17, 2014
Test end date	: October 17, 2014
Final report(draft) submission date	: November 3, 2014
Study end date	: November 4, 2014

3. Materials & Methods

3.1 Test substance and control substance (washing agent)

3.1.1 Test substance (Figure 3.)

Name of substance : Silver ion sterilizer

Origin of supply: CNL Co., Ltd.

Date of production: September 1, 2014

Installation date: October 6, 2014

Installed device: Silver ion water producing device set 1

Storing condition during test period: room temperature[(1-30)℃], to be used on the same day it is produced

Appearance and state: Transparent liquid when produced as silver ion water

Effective life period: Until silver plates perish (provided by sponsor)

Disposal of remaining test substance: Discard silver ion water and return producing

Notes for treating/disposing: Keep the device from direct contact of water

3.1.2 Control Substance (Washing agent)

Name of substance: Sterilized distilled water (main water used)

Producer: Dai Han Pharm Co., Ltd.

3.2 Fabrication of Test substance

We used the test substance generated from the silver ion sterilizer that the sponsor installed(device) and set(standard values) without additional preparation.

3.3 Analyses on Test substance

We decided not to conduct a separate analysis on the concentration, safety and

homogeneity of the test substance and test substance preparations after discussing with the test sponsor.

3.4 Test specifications

Type and species: Yac:NZW(KBL), Rabbit, SPF

Origin of supply: Cheonan Yonam College (313 Yonam-ro, Seonghwan-eup, Seobuk-gu, Cheonan-si, Chungnam)

Gender and number of animals when adopted : 6 males

Age and weight of animals when adopted :

About 3 months old, 1803.4 ~ 1980.4g

Gender and number of animals at the time of application : 6 males

Age and weight of animals at the time of application : About 3 months old, 2095.0~2312.8g

3.4.1 Reason for selecting the test specifications

We selected NZW rabbits since they are widely used in skin irritation tests, and thus there are abundant basic data accumulated that may be used for comparison.

3.4.2 Quarantine and purification

We examined the appearance and health conditions of all the animals when adopting them, and then we observed their weight changes and general health conditions after 8 days of quarantine and purification, and then used the healthy ones for the test.

3.4.3 Entity identification

We marked animal numbers with an oil-based pen on the left earflaps of the rabbits during the purification period, and on the their right earflaps during the testing period, and also attached ID cards to the cage.

3.5 Raising environment

3.5.1 Number of animals rooms

Rabbit raising room 5

3.5.2 Environmental conditions

Temperature: (20 ± 3)℃

Relative humidity: (50 ± 20) % R.H.

Number of times of ventilation: (10 ~ 20) times/h

Lighting cycle: Bright condition 12 hours (08:00 ~ 20:00)

Dark condition 12 hours (20:00 ~ 08:00)

Illumination: (150 ~ 300) Lux

Cage type: Stainless steel wire cage

Cage size: (470W x 405D x 600H)mm

Capacity per cage: 1 rabbit

The temperature and humidity of the animal room was measured every 30 minutes by an automatic temperature humidity measuring device, and environmental conditions such as the illumination was measured according to the standard operation guidelines. As a result of measuring the environment of the animal room, there were no factors affecting the test.

3.5.3 Feed and drinking water supply

Radiation sterilized feed for rabbits for testing [Purina, Korea] was used as the feed, and R/O water was provided as free drinking water.

3.5.4 Feed and drinking water inspection

We inspected the feed based on the analysis report that had been prepared under regular inspections by the manufacturer of the feed that we received from the feed supplier, and we inspected the drinking water for any effects on the test based on the

regular inspections according to the standard operation guidelines of the Health Care Research Laboratory of Korea Testing and Research Institute, but found none.

3.6. Test method

3.6.1 Group configuration

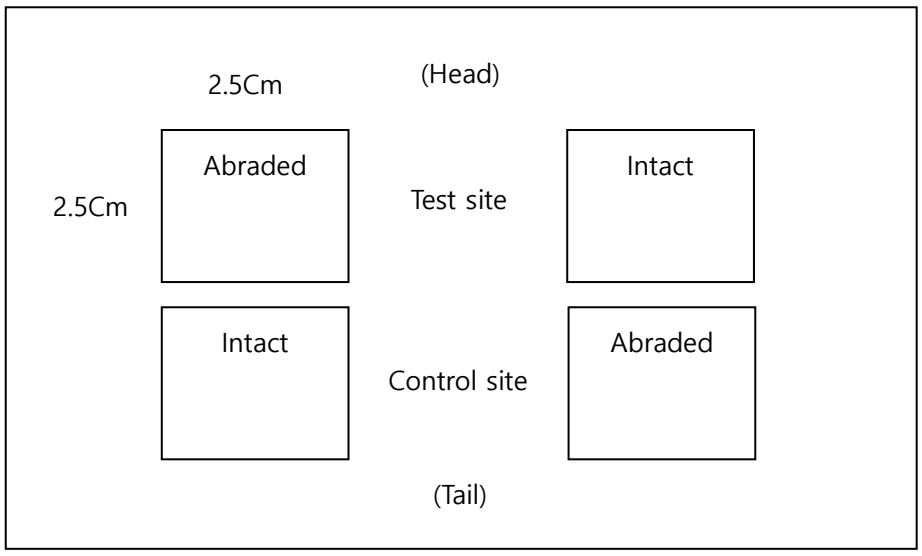
Test group	Gender	Animal number	Number of animals	Amount applied	Application route to subject area (per animal)
T1	Male	1101-1106	6	Control substance: 0.5mL Test substance: 0.5mL	Skin

3.6.2 Treatment before Application

We waxed the back area before 24 hours of application, and then right before the application, we marked two test sites and two control sites, each having a size area of (2.5 x 2.5)cm^2. We then designated the test sites and control sites as abraded areas and intact areas, respectively, and then abraded the abraded areas such that only the epidermis and not the corium is damaged.

3.6.3 Application of test substance

We applied the test substance to a gauze [(2.5 x 2.5)cm2] uniformly. We then attached the gauze with the test substance applied to an upper abraded area and an intact area. For each of the lower control sites, we applied 0.5mL of sterilized distilled water to a gauze and attached the gauze to the lower control sites. We then carefully fixated the application areas with nonirritating tape(Tegarderm, 3M) and elastic support bandage(Coban, 3M). We removed the gauzes after 24 hours, and then gently washed what was left on the skin using the washing agent.



Skin area of application

3.7 Observation items

3.7.1 General symptoms

We observed all the animals once a day, and after 72 hours that is the time of end of observation, we euthanized them.

3.7.2 Weight changes

We measured the weights of the animals when adopted, just before applying the test substance, and after 72 hours, that is the time of end of observation.

3.7.3 Observation on skin reaction

After 24 hours and 72 hours since application of the test substance, we observed the condition of erythema, eschar and edema according to [Table A]/

3.7.4 Photographing

After 24 hours and 72 hours since application of the test substance, we selected one entity from each group that has representativeness, and then attached a label having the test number and the name of test substance, and photographed them.

3.7.5 Evaluation and judgment on skin reactions

We evaluated skin reaction according to [Table A], and computed the Primary Irritation Index (P.I.I.). Skin irritation was judged according to [Table B].

$$\text{P.I.I. (Primary Irritation Index)} = \frac{\text{Sum of 24 and 72 h readings}}{\text{(No. of test sites=12) x (scoring intervals =2)}}$$

[Table A]

(1) Formation of erythema and eschar
No erythema at all.....0
Very slight erythema (barely noticeable by unaided eye).....1
Clear erythema.....2
Slightly severe erythema.....3
Severe erythema(of carrot color) and a slight eschar.....4
Highest score.....4
(2) Formation of edema
No edema at all.....0
Very slight edema(barely noticeable by unaided eye).....1
Slight edema(clearly swollen and thus marginal area is distinguishable).....2
Moderate edema(swollen about 1mm).....3
Severe edema(swollen at least 1mm and expanded outside the exposed area).....4
Highest score.....4

[Table B] Skin irritation score table

Primary Irritation Index (P.I.I.)	Type
0.0 – 0.5	Non irritant
0.6 – 2.0	Mild irritant
2.1 – 5.0	Moderate irritant
5.1 – 8.0	Severe irritant

4. Results

4.1 Mortality and general symptoms (Table 1)

There were no dead animals or abnormal findings caused by the application of test substance during the test period.

4.2 Weight changes (Table 2)

All the animals showed normal increase of weight.

4.3 Observation on applied area (Table 3, Figure 1 -2)

There were no skin irritation observed at 24 and 72 hours after the application of the test substance.

5. Discussion & conclusion

In order to evaluate skin irritation on rabbits by silver ion water generated from a silver ion sterilizer, we applied the test substance on the rabbits' skin, and at 24 hours and 72 hours after the application, we evaluated the mortality, general symptoms, weight changes, and skin irritation.

There were no dead animals or abnormal findings caused by the application of test substance during the test period. All the animals showed normal increase of weight.

No skin irritation was observed after 24 hours and 72 hours since application of the test substance. The evaluation result by Draize calculating method: Primary Irritation Index(P.I.I.) was '0.0'.

According to the above skin irritation test on rabbits, silver ion sterilizers are considered to be 'non-irritant' matter.

6. References

- Ministry of Food & Drug Safety Announcement no. 2014-136 (Jul. 30, 2014) "Toxicity Test Standards of Drugs etc." [Appendix 8] ② Skin irritation test

- Ministry of Food & Drug Safety Announcement no. 2014-67 (Feb., 12, 2014) "Nonclinical Test Management Standards"

-OECD Principle of Good Laboratory Practice, ENV/MC/CHEM (98)17 (as revised in 1997)

-Draize J.H. (1959): Dermal toxicity, Assoc. Food and Drug officials, U.S. Appraisal of the Safety of Chemicals in Food, Drugs, and Cosmetics, Texas state Dept. of Health Austin, pp 46-59, Texas.

-Draize J.H., Woodard G. and Calvery H.O. (1944) : Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Ther., 82:377-390.

7. Tables (Group summary)

Table 1. Mortality rate and clinical signs

Animal number	Days after application				Mortality
	0	1	2	3	
1101	N	N	N	N	0/6[a]
1102	N	N	N	N	
1103	N	N	N	N	
1104	N	N	N	N	
1105	N	N	N	N	
1106	N	N	N	N	

N : Normal

[a] : Number of dead animals/Number of total animals

Table 2. Body weight

Unit (g)

Animal number	Hours after application		Weight gains
	0 h	72 h	
1101	2095.0	2139.9	44.9
1102	2153.3	2233.4	80.1
1103	2162.3	2225.7	63.4
1104	2312.8	2385.9	73.1
1105	2299.6	2408.5	108.9
1106	2275.4	2308.9	33.5
Mean	2216.4	2283.7	67.3
S.D.	90.9	103.2	26.8

S.D. : Standard deviation

Table 3. Evaluation of skin irritation

Sites	Control Sites							
Change	Erythema & Eschar				Edema			
Phases[1] / Animal number	Intact		Abraded		Intact		Abraded	
	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
1101	0	0	0	0	0	0	0	0
1102	0	0	0	0	0	0	0	0
1103	0	0	0	0	0	0	0	0
1104	0	0	0	0	0	0	0	0
1105	0	0	0	0	0	0	0	0
1106	0	0	0	0	0	0	0	0
Total	0	0	0	0	0	0	0	0
Sum[2] (S)	0							
P.I.I. (S/24*)	0.0							

Sites	Test Sites							
Change	Erythema & Eschar				Edema			
Phases[1] / Animal number	Intact		Abraded		Intact		Abraded	
	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
1101	0	0	0	0	0	0	0	0
1102	0	0	0	0	0	0	0	0
1103	0	0	0	0	0	0	0	0
1104	0	0	0	0	0	0	0	0
1105	0	0	0	0	0	0	0	0
1106	0	0	0	0	0	0	0	0
Total	0	0	0	0	0	0	0	0
Sum[2] (S)	0							
P.I.I. (S/24*)	0.0							

[1] : Time after topical application

[2] : Sum of 24 and 72 h readings

* : (No. of test sites = 12) × (scoring intervals = 2)

8. Figures

Figure 1. Skin photograph at 24 hours after application of test substance

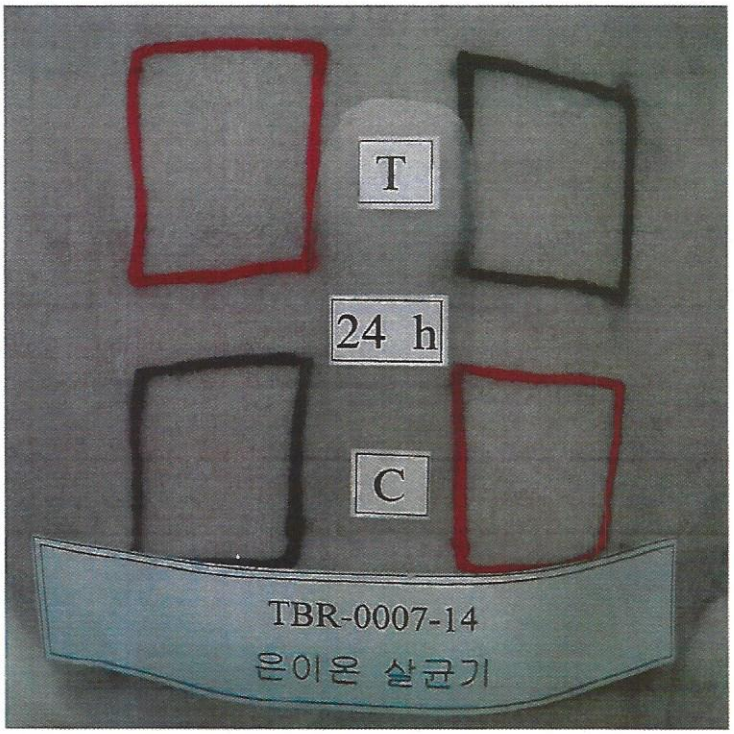


Figure 2. Skin photograph at 72 hours after application of test substance

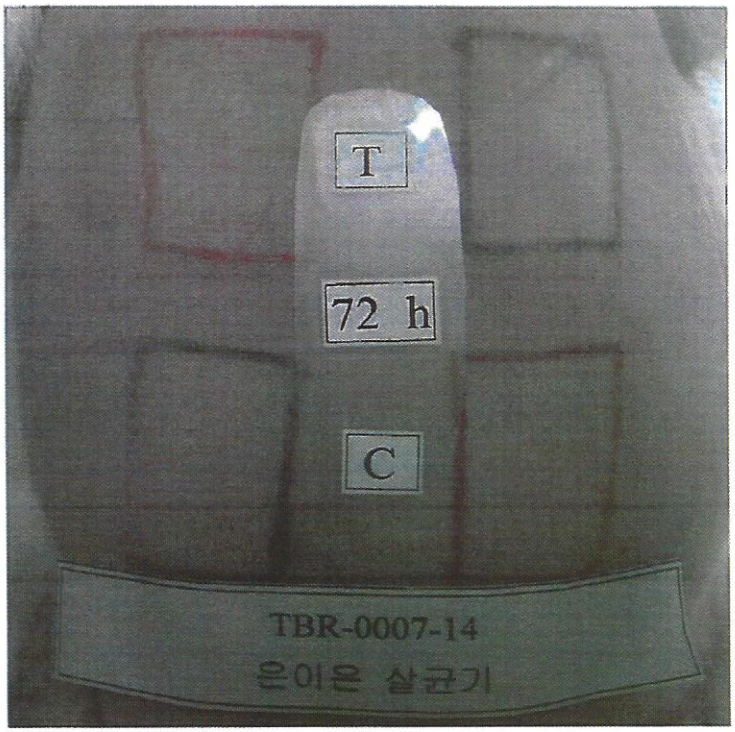


Figure 3. Test substance